

Effect of Purple Sweet Potato (*Ipomoea Batatas L.*) Extract on Glutathione Peroxidase (GPx) Activities in Hepatic House Mice (*Mus musculus*) After Maximum Physical Exercise

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ABSTRACT

Background: Physical exercise can improve antioxidant defense system of organisms. But long and heavy exercise can disrupt the balance of oxidant-antioxidant. Low glutathione peroxidase is associated with free radicals. Plant purple sweet potatoes (*Ipomoea batatas L.*) contain anthocyanins high enough that act as antioxidants. This study aimed to determine the effect of extracts of tubers of purple sweet potato (*Ipomoea Batatas L.*) on the activity of glutathione peroxidase (GPx) liver of mice (*Mus Musculus*) treated with maximal physical exercise.

Subjects and Method: This was a true experimental study. This conducted with the design of the control group post-test only in vivo on 24 white male mice (*Mus musculus*), DD Webster strain. Data analysis was using SPSS software 19. Statistical testing decision taken 5% significance level ($p=0.05$).

Results: The results showed that the average enzyme activity of GPx in the P5 is the highest enzyme activity GPx (mean=19:39 \pm SD=7:06, $p=0.024$), which means there are significant differences in the enzyme activity of GPx values between groups.

Conclusion: This study showed that the extract could increase the activity of GPx hepatic enzymes mice significantly.

Keywords: physical exercise maximum, free radicals, purple sweet potato, anthocyanin, GPx

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BACKGROUND

Physical exercise and or sport activity are efforts to increase the degree of health (Depkes, 2014). American College and Sports Medicine recommends exercises to attain cardiorespiratory fitness and body trimness by regarding frequency, intensity, duration, and types of activity (Sastradipradja, 2014). Although exercise increases organism's antioxidant defense system, however, long period and heavy exercise will violate the balance between oxidant and antioxidant (Escribano et al., 2010).

During maximal physical exercise, free radicals' secretion especially superoxide may increase in mitochondria or

energy centers within cells, whenever body cells use oxygen to produce energy, body cell may form reactive molecules (easily react) called free radicals (Daniel et al., 2010). Free radicals molecules are not stable because of electron deficiency on one of its atom. The unstable molecule will actively seek for electron pair for the atom with electron deficiency (Agarwal, 2005). It will very actively react with the existing molecules around. The free radicals reaction with the existing molecules within body often harm body cell (Cooper, 2001).

Body has protection mechanism which neutralize created free radicals, among others with the existence of superoxide dis-

mutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes (Winarsi, 2007). Delmas-Beauvieux et al (1996) reports that glutathione peroxidase enzyme more strongly decomposes H_2O_2 compared to catalase enzyme. Activity of glutathione peroxidase enzyme is able to reduce 70% of organic peroxide and more than 90% of H_2O_2 (Winarsi, 2007).

Within liver and red blood cell exist high concentration of glutathione peroxidase, whereas heart, kidney, lungs, adrenal, stomach, and adipose tissue contain moderate level of glutathione peroxidase, low level of glutathione peroxidase is often found in brain, muscle, testicle, eye lens (Sugianto, 2011). The activity of the enzyme may also be induced by isoflavones secondary antioxidant (Chen *et al*, 2002).

One of flavonoid components from plant that may function as antioxidant is natural color substance which is called anthocyanin (Simanjuntak, 2012). Based on his study Sugianto (2011) concludes that the administration of red pomegranate (*Punica granatum*) that contains anthocyanin may increase glutathione peroxidase in the blood of mice (*Mus musculus*) with maximal physical exercise (Sugianto, 2011).

Based on study result of Agriculture Faculty Udayana University Bali it found purple sweet potato plant (*Ipomoea batatas L.*) whose tubers contain quite high anthocyanin that is about 110mg-210 mg/100 gram (Suprpta, 2004). The administration of local Balinese purple sweet potato extract which is either unprocessed or in a form of syrup, may protect liver tissue from free radicals as the result of maximal physical exercise on mice (Jawi, 2007).

SUBJECTS AND METHOD

Research Design

The study was purely experimental with research design post-test only, control group

design. Sample was divided into 6 groups each consisted of 5 mice. P1 as a control which did not endure any treatment at all only eat and drink in *ad libitum*. P2 endured maximal physical exercise which was swimming for 60 minutes and 0.5 ml/ mouse/day of aqua bidest orally. P3 got 0.5 ml/ mouse/ day of purple sweet potato tuber extract orally. P4 got maximal physical exercise which was swimming for 60 minutes and 0.5 ml/mouse/day of purple sweet potato tuber extract orally. P5 got maximal physical exercise which was swimming for 60 minutes and 1 ml/ mouse/ day of purple sweet potato tuber extract orally. got maximal physical exercise which was swimming for 60 minutes and 1.5 ml/ mouse/ day of purple sweet potato tuber extract orally. The drenching of aqua bidest and purple sweet potato tuber extract were conducted 3 hours after swimming. All treatments were conducted for 14 days.

Extract Making

The tubers of purple sweet potato were rinsed with clean water peeled out and chopped crosswise with 2-2.5 cm of thickness. The chopped tubers were steamed for ± 15 minutes. Afterward the tubers were cooled down and put in a glass jar to be fermented by adding tape starter (wine yeast) which was bought in traditional market. The fermentation was conducted for 36 hours. The result of fermentation (tapai) was mixed with clean drinking water with comparison 1kg of fermented purple sweet potato (tapai) added by 1 liter of water and then blended and screened with gauze. The screening result was given to mice. The level of anthocyanin was measured by measuring the absorbance of purple color of anthocyanin by using spectrophotometer on 520 nm and 720 nm. From the measuring it was obtained that the level of anthocyanin on fresh purple sweet potato

was 9,984 mg/100 g and on fermented tubers was 89,666 mg/100 g.

Enzyme Activity Measurement

After liver was separated from the mouse body, liver was rinsed with NaCl 0.9%. It was weighed 0.1 g and then put into homogenizer tube and kept in the cool box with ice in it. Liver was crushed using homogenizer, and it was conducted in a cool box with ice in it. The crushed liver was added with 0.2 ml of cold assay buffer solution. Afterward, it was centrifuged on 10.000 x gravitation for 15 minutes in the temperature 4°C. The produced supernatant would be used for the test. The examination of liver GPx was conducted with Glutathione Peroxidase Activity Colorimetric Assay Kit from Biovision and using spectrophotometer.

Data Analysis

The data was not normally distributed therefore Kruskal-Wallis was used. To observe the existence of differences between control

group with treatment group Post-Hoc test was conducted. All data analysis was conducted by using SPSS 19 software. In this study for statistics test decision the significance level obtained was 5% ($p=0.05$) which was considered significant

RESULTS

From the study result it obtained measuring data for Glutathione Peroxidase enzyme activity on mice's liver after the treatment, as it is presented in Table 1. Based on Table 1 it is discovered that the mean of GPx enzyme activity on P5 group is the highest GPx enzyme activity that is 19.39 ± 7.06 , an then consecutively followed by P4 (7.05 ± 8.19), P1 (6.58 ± 7.04), P3 (4.50 ± 4.31), P6 (3.03 ± 1.98), and P2 (1.84 ± 0.92) is the lowest GPx enzyme activity.

On Kruskal-Wallis test, it obtained the value of $p=0.024$ that means there is significant difference of GPX enzyme activity value among groups since $p < 0.05$.

Table 1. Result of Glutathione Peroxidase enzyme activity value mean (GPx) on mice liver (*Mus musculus L.*) after 14 days of treatment

Treatment Group	Number of Mice	Mean \pm SD (mU/ml)	p
(P1) Mean	4	6.58 ± 7.04	*0.024
(P2) MPE	4	1.84 ± 0.92	
(P3) 0.5 ml extract	4	4.50 ± 4.31	
(P4) MPE + 0.5 ml extract	4	7.05 ± 8.19	
(P5) MPE + 1 ml extract	4	19.39 ± 7.06	
(P6) MPE + 1.5 ml extract	4	3.03 ± 1.98	

Information :

MPE : Maximal Physical Exercise

* $p \leq 0.05$ the significance is different compared to positive control

DISCUSSION

In accordance with liver function as detoxification organ therefore damage on liver will lead to intoxication within the body. The early signs of liver function disorder

can be conducted by measuring the value of enzyme activity (Musthofiyah, 2008).

This study found a significant different among the groups, in which in P2 there was significant reduction compared to control group (P1). It was because P2 was

group that only received treatment of maximal physical exercise in 60 minutes for 14 days. Oxygen consumption by body during heavy exercise may increase up to two times or more (Thirumalai et al.) it is in accordance with the study conducted by Daniel et al. There was an increase on the activity level of SOD, CAT, and reduce GSH on liver after doing maximal physical exercise. Similarly with the study conducted by Lima et al., on exercise group it boosts the reduction of glutathione (GSH), increases MnSOD, and reduces lipid peroxidase on liver mitochondria (Lima et al., 2013).

Under a high stress, within muscle fiber occurs the increase of oxygen use above normal need. The extraordinary oxygen needs may generate free radicals release in mitochondria that lead to increasing use of GPx within the body. It will reduce the amount of GPx within the body (Sugianto, 2011).

It was discovered from the data that P5 group that received treatment in a form of maximal physical exercise and the administration of purple sweet potato tubers extract had GPx enzyme activity value (19.39 ± 7.06) which was significantly different from P1 group (6.58 ± 7.04), P3 (4.50 ± 4.31), and P6 (3.03 ± 1.98), whereas from P4 (4.50 ± 4.31), it had different value however it was not significant. On P5 there was significant increase on GPx enzyme activity that can be seen on table 1 and table 2, it is in accordance with the study conducted by Sugianto (2011) the administration of red pomegranate juice (*Punica granatum*) may increase the level of blood glutathione Peroxidase on mice (*Mus musculus*) with maximal physical activity. The study conducted by Hou et al (2010) found that the anthocyanin extract from black rice (*Oryza sativa L. Japonica*) was able to increase the level of blood glutathione peroxidase on the

liver of male mice which were induced by alcohol for 45 days.

From the result of the study it can be concluded that the administration of purple sweet potato tubers extract in moderate dosage will increase GPx enzyme activity. In this study it found that the better dosage was 1 ml of purple sweet potato tubers extract.

Based on the study it can be concluded that the administration of purple sweet potato tubers extract in moderate dosage would increase enzyme GPx activity on the liver of mice. The study found that the better dosage was 1 ml of purple sweet potato tubers extract. In which there was significant difference since $p < 0.005$.

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